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**GLP-1 and IGF-I levels are elevated in late infancy in low birthweight infants,
independently of GLP-1 receptor polymorphisms and neonatal nutrition**

Marta Díaz, MD, PhD ^{*#}, Cristina García-Beltran, MD ^{*#}, Abel López-Bermejo, MD, PhD [‡],
Francis de Zegher, MD, PhD [£], Lourdes Ibáñez, MD, PhD ^{*#}

* Endocrinology, Pediatric Research Institute Sant Joan de Déu, University of Barcelona, 08950 Esplugues, Barcelona, Spain

Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), ISCIII, 28029 Madrid, Spain

‡ Department of Pediatrics, Hospital Dr. Josep Trueta & Girona Institute for Biomedical Research, 17007 Girona, Spain

£ Department of Development & Regeneration, University of Leuven, 3000 Leuven, Belgium

Corresponding Author: Lourdes Ibáñez, MD, PhD; libanez@hsjdbcn.org. Hospital Sant Joan de Déu, University of Barcelona. Passeig de Sant Joan de Déu, 2; 08950 Esplugues, Barcelona, Spain. Phone: +34 93 2804000; ext. 4424; Fax: +34 93 2033959

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Abstract

Low birth weight followed by rapid postnatal weight gain is associated with increased risks for obesity and diabetes in adulthood. Modulation of glucagon-like-peptide 1 (GLP-1) secretion by (epi)genetic mechanisms or nutrition may influence in part this risk. Formula fed infants born small-for-gestational-age (SGA) have higher circulating GLP-1 at age 4 months than breastfed SGA or appropriate-for-gestational-age (AGA) infants. Here, we assessed GLP-1 concentrations in healthy AGA (n=149) and SGA (n=107) subjects at age 12 months, and their association with endocrine-metabolic and body composition parameters and GLP-1 receptor (*GLP-1R*) rs6923761 and rs3765467 polymorphisms. At birth, cord GLP-1 concentrations were comparable in AGA and SGA infants. At age 12 months, insulin-like growth factor I (IGF-I) and GLP-1 levels were higher than at birth; SGA infants displayed higher IGF-I and GLP-1 concentrations than AGA infants (both $p < 0.001$), that were unrelated to neonatal nutrition or *GLP-1R* genotype, and that were paralleled by a significant increase in weight Z-score ($p < 0.001$ vs AGA). In conclusion, SGA infants have augmented IGF-I and prefeeding GLP-1 concentrations in late infancy. Increased GLP-1 levels may impair hypothalamic and/or peripheral *GLP-1R* signaling, exert long-term negative effects on the hypothalamic nuclei regulating energy homeostasis and increase the risks for obesity and diabetes.

Introduction

Low birth weight followed by early postnatal weight gain has been associated with increased metabolic risk in adulthood, including obesity, type 2 diabetes and cardiovascular disease (1,2). Early nutrition may influence in part this risk (1,3,4), one potential mechanism being through modulation of glucagon-like peptide 1 (GLP-1) secretion, an incretin that improves peripheral insulin sensitivity, and regulates satiety as well as energy expenditure via the hypothalamic activation of the GLP-1 receptor (*GLP-1R*) (5-7).

Several nonsynonymous single nucleotide polymorphisms (SNP) at *GLP-1R* potentially modifying GLP-1 binding affinity have been identified (8). For example, the missense variants rs6923761 and rs3765467 alter insulin secretion in response to GLP-1 infusion in nondiabetic subjects (9) and impact the effectiveness of treatments targeted to *GLP-1R* in individuals with type 2 diabetes (10,11). However, there are no data on the relationships between those variants and the endocrine-metabolic status in early life.

We have recently shown that formula-fed infants born small-for-gestational age (SGA) have increased GLP-1 levels at age 4 months, as compared to both breastfed SGA infants and to breastfed infants born appropriate-for-GA (AGA) (5). Here, we assessed whether GLP-1 concentrations remain elevated in late infancy, and whether they relate to endocrine-metabolic markers, body composition, and *GLP-1R* rs6923761/rs3765467 genotype.

Subjects and Methods

Study Population and Design

The study population consisted of 256 newborns (133 girls, 123 boys), who were recruited into a previous longitudinal study assessing DNA methylation in placenta and cord blood of SGA vs AGA infants and its association with endocrine-metabolic and body composition parameters (12) (Supplemental Figure 1).

Inclusion criteria were: 1) maternally uncomplicated, singleton pregnancy with delivery at term (37-42 weeks); 2) birthweight between 2.9 and 3.8 kg for AGA (range, -1.0 and +1.0 SD), and between 1.9 and 2.6 kg for SGA (<-2 SD); 3) exclusive breastfeeding or formula feeding in the first 4 months; 4) cord serum and blood samples at 12 months available for GLP-1 and *GLP-1R* polymorphism assessment; 5) written informed consent. Exclusion criteria were: maternal hypertension, preeclampsia, gestational diabetes, alcohol or drug abuse, congenital malformations and complications at birth.

Maternal age at delivery, parity, pregestational weight and body mass index [BMI, weight (Kg)/height (m²)], were retrieved from maternal clinical records. Gestational age was calculated according to the last menses and confirmed by first-trimester ultrasound.

Assessments

Weight and length were measured at birth and at 12 months (12) and Z-scores derived. Blood samples were obtained at birth (from the umbilical cord before placental separation), and in prefeeding state at age 12 months. Whole blood collected in EDTA tubes was used for DNA extraction. Serum samples were also obtained and kept at -80°C.

Serum glucose was measured by the glucose oxidase method. Insulin and insulin-like growth factor (IGF-I) were assessed by immunochemiluminiscence (DPC IMMULITE 2500, Siemens, Germany); intra- and inter-assay coefficients of variation (CVs) were <10% (5). Insulin resistance was estimated with the homeostatic model assessment [fasting insulin (mU l⁻¹) X fasting glucose (mmol l⁻¹)/22.5]. Total serum GLP-1 was assessed by ELISA (Millipore, Billerica, MA, USA) (5). The antibody pair in the assay binds to GLP-1 (7-36) and (9-36) and has no cross-reactivity with GLP-2, GIP, glucagon or oxyntomodulin. The intra- and inter-assay CVs were <2% and <10% respectively, and the detection limit was 1.5

pM. High-molecular-weight (HMW) adiponectin was assessed by ELISA (Millipore St. Louis, MO, USA); intra- and inter-assay CVs being <9%.

Body composition was assessed at age 15 days and at 12 months, by dual X-ray absorptiometry (DXA) with a Lunar Prodigy coupled to Lunar software (Lunar Corp, Madison, WI), adapted for infants (5,12). CVs were less than 3% for fat and lean mass.

Genetic analysis

Genomic DNA was extracted from whole blood, as described (12). Genotyping of rs6923761 and rs3765467 SNPs in the *GLP-1R* gene was performed by 5'-nuclease allelic discrimination assay (ID C_25615272 for rs6923761; a custom Taqman design service for rs3765467, Applied Biosystems). Twenty ng of DNA was amplified after 40 cycles with an initial denaturation of 10 min at 92°C. The cycle program consisted of 30s denaturation at 92°C and 30s annealing and extension at 60°C.

Statistics and Ethics

Statistical analysis was performed with SPSS software 23.0 (Inc Chicago, IL). Normally distributed variables were analyzed with a two-tailed student t-test. Non-parametric variables were analyzed with the Mann-Whitney U test. Genotype frequencies were tested for Hardy-Weinberg equilibrium using the chi-square test and the effect of genotype was assessed using a dominant model (wild type homozygotes vs individuals with one or more copies of the minor allele). The associations between circulating GLP-1 and auxological, metabolic and body composition parameters were assessed by linear regression and multiple regression analysis. The significance was set at $p < 0.05$.

The study was approved by the Institutional Review Board of Hospital Sant Joan de Déu, Barcelona. Informed consent was obtained from parents, including permission for genotyping and for linking phenotype and genotype in an anonymized fashion.

Results

Maternal and offspring endocrine-metabolic and body composition data

Table 1 depicts maternal variables and longitudinal infant data by birthweight subgroup. At birth, cord GLP-1 was similar in AGA and SGA infants, whereas lean mass, total and abdominal fat, and circulating levels of IGF-I were lower in SGA newborns, as expected (4,5,13). At age 12 months, SGA infants remained lighter and shorter, had less fat and lean mass, but higher IGF-I and GLP-1 concentrations than AGA infants. Weight Z-score, IGF-I and GLP-1 concentrations increased significantly in SGA infants between 0 and 12 months (Figure 1). At 12 months, GLP-1 levels were similar in SGA infants who were breastfed or formulafed from birth to 4 months (Supplemental Figure 2). Body composition and IGF-I, but not circulating GLP-1, exhibited gender-dependent differences (Supplemental Figure 3).

GLP-1R rs6923761 and rs3765467 genotype: relation to clinical, biochemical and body composition parameters

Allele frequencies for both rs6923761 and rs3765467 were in Hardy-Weiberg equilibrium and did not differ between AGA and SGA infants (data not shown).

There were no readily detectable polymorphism effects within AGA and SGA subgroups. At age 12 months, SGA infants with a GG genotype in both polymorphisms had less fat than GG AGA infants; SGA subjects displaying a GG haplotype in rs3765467 showed also higher IGF-I and GLP-1 levels than AGA infants (Supplemental Tables 1a & 1b).

Correlations and multiple regression analysis

The two most significant correlations ($R^2 > 0.3$) are depicted in the bottom panels of Figure 1. Multivariate analysis showed that body weight at 12 months was independently explained by neonatal lean mass and by cord GLP-1 levels, whereas total fat at 12 months was predicted by cord IGF-1 and GLP-1 levels (Supplemental Table 2).

Discussion

To our knowledge, this is the first longitudinal study disclosing that SGA infants display in late infancy higher serum IGF-I and GLP-1 levels than AGA infants; the differences in circulating GLP-1 are not influenced by early nutrition or by genetic variants in *GLP-1R*.

The ontogeny of GLP-1 in a rat model has disclosed that this incretin increases gradually from birth through infancy and decreases in adulthood (14). These data fit with our findings, as the supra-adult GLP-1 concentrations present at age 4 months (5) persisted throughout infancy, experiencing a further rise in SGA infants. These comparatively higher GLP-1 levels may indicate an augmented rate of pancreatic endogenous production to promote beta cell mass and function (15-17), and thus preserve insulin sensitivity through infancy, while spontaneous catch-up is still ongoing. The increased IGF-I levels in SGA infants and the positive association between GLP-1, IGF-I and weight z-score increase over 12 months suggests that GLP-1-mediated activation of IGF-I receptor signaling could be among the mediators of this catch-up (17).

An epigenetic-dependent hypersecretion of intestinal GLP-1 may also partly account for the higher GLP-1 concentrations in SGA subjects. Dietary fatty acids induce GLP-1 secretion through binding and activation of the G-protein-coupled receptor *GPR120* (18). Recently, we reported altered *GPR120* methylation and expression patterns in placenta and

cord blood of SGA infants (12), reflecting the importance of fetal epigenetic programming in the control of energy homeostasis through the gut-brain axis.

The increased GLP-1 concentrations in SGA infants could also aim at counteracting a decrease in *GLP-1R* signaling, a process partly regulated by DNA methylation (19). Although genetic variants of *GLP-1R* can also modulate GLP-1 action (9), we did not find associations between *GLP-1R* polymorphisms and studied variables to support this notion.

Regardless of the mechanism(s) involved, elevated GLP-1 levels in late infancy may impair hypothalamic and/or peripheral *GLP-1R* signaling and thus increase the risks for obesity and diabetes. Along these lines, catch-up SGA children already display impaired beta cell function at age 4 years in the presence of preserved insulin sensitivity (20).

The main study limitation is the relatively small population; the strengths include the longitudinal design and the co-availability of body composition and endocrine-metabolic data.

In conclusion, SGA infants have augmented IGF-I and prefeeding GLP-1 concentrations in late infancy. The increased GLP-1 levels may impair hypothalamic and/or peripheral *GLP-1R* signaling, exert long-term negative effects on hypothalamic nuclei regulating energy homeostasis and subsequently increase the risks for obesity and diabetes.

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Conflict of Interest: The authors declare no conflict of interest.

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Figure Legends**Figure 1**

Box plots (median and interquartile ranges) of circulating GLP-1 at 0 and at 12 months (upper left) and 0-12 month changes (upper right) in appropriate (AGA, n=149) and small-for-gestational-age (SGA, n=107) infants. Middle panels depict the 0-12-month changes in weight Z-score (left) and in circulating IGF-I levels (right). Lower panels depict Pearson correlations between 0-12 month changes in circulating GLP-1 and changes in Weight Z-score (left) and in circulating IGF-I (right) in the whole AGA and SGA population (n=256).

* $P < 0.001$ versus birth.

Table 1. Maternal and infant data by birthweight subgroups at birth and at age 12 months.

	Birth		12 months		Δ 0-12 months	
	AGA n=149	SGA n=107	AGA n=149	SGA n=107	AGA n=149	SGA n=107
Mothers						
Age (yr)	33.1 ± 0.5	32.6 ± 0.7	--	--	--	--
Pre-gestational weight (Kg)	64.3 ± 1.2	56.3 ± 1.1 ^c	--	--	--	--
Pre-gestational BMI (Kg/m ²)	24.7 ± 0.5	21.8 ± 0.4 ^c	--	--	--	--
Primiparous (%)	53	29 ^c	--	--	--	--
Caesarean section (%)	27	46 ^b	--	--	--	--
Smoking (%)	13	34 ^c	--	--	--	--
Infants						
Gestational age (Wk)	39.7 ± 0.1	38.6 ± 0.1 ^c	--	--	--	--
Females (%)	50	54	--	--	--	--
Breastfeeding (%)	78	44 ^c	--	--	--	--
Weight (Kg)	3.30 ± 0.03	2.28 ± 0.02 ^c	9.76 ± 0.11	9.02 ± 0.11 ^c	6.46 ± 0.10	6.74 ± 0.11
Weight SDS	-0.05 ± 0.05	-2.31 ± 0.04 ^c	-0.03 ± 0.10	-0.76 ± 0.10 ^c	0.01 ± 0.11	1.52 ± 0.11 ^c
Length (cm)	49.9 ± 0.2	45.7 ± 0.2 ^c	74.9 ± 0.3	73.8 ± 0.4 ^a	27.1 ± 0.9	28.9 ± 0.8
Length SDS	-0.05 ± 0.07	-1.79 ± 0.08 ^c	0.09 ± 0.15	-0.36 ± 0.17 ^a	0.1 ± 0.2	1.4 ± 0.2 ^c
Glucose (mg/dL) [*]	--	--	83 ± 1	83 ± 1	--	--
Insulin (mIU/mL) [†]	--	--	3.0 ± 0.3	2.7 ± 0.3	--	--
HOMA-IR [†]	--	--	0.6 ± 0.1	0.6 ± 0.1	--	--
HMW adiponectin (mg/L) [†]	30 ± 1	29 ± 2	17 ± 1	19 ± 1	-13 ± 1	-10 ± 2
IGF-I (ng/mL) [†]	55 ± 2	41 ± 3 ^c	53 ± 2	88 ± 7 ^c	-1 ± 3	47 ± 8 ^c
GLP-1 (pmol/L) [†]	17 ± 1	17 ± 1	30 ± 2	54 ± 2 ^c	13 ± 2	37 ± 2 ^c
Postnatal age at DXA (days)	15 ± 0	16 ± 0	391 ± 3	392 ± 3	376 ± 3	376 ± 3
Lean mass (Kg) [‡]	3.02 ± 0.03	2.36 ± 0.03 ^c	6.71 ± 0.07	6.01 ± 0.11 ^c	3.69 ± 0.06	3.64 ± 0.11
Total fat mass (Kg) [‡]	0.75 ± 0.02	0.48 ± 0.02 ^c	3.54 ± 0.06	3.24 ± 0.09 ^b	2.81 ± 0.07	2.76 ± 0.07
Abdominal fat mass (g) [‡]	37 ± 2	24 ± 1 ^c	187 ± 6	178 ± 6	150 ± 6	153 ± 6

Values are mean ± SEM.

AGA, appropriate-for-gestational age; SGA, small-for-gestational age; BMI, body mass index; HOMA-IR, homeostatic model assessment-insulin resistance; IGF-I, insulin-like growth factor-I; HMW-adiponectin, high-molecular-weight adiponectin; GLP-1, glucagon-like peptide-1

^{*} Circulating levels measured in cord blood

[†] Circulating levels measured in cord serum

[‡] By dual X-ray absorptiometry (DXA)

^a p<.05, ^b p<.01 and ^c p<.001 between AGA and SGA subgroups at baseline, at 12 months, and for changes from birth to 12 months

Figure 1